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THE EFFECT OF CONTINUED DESICCATION ON THE EXPULSION OF ASCOSPORES OF ENDOTHIA PARASITICA

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The importance of ascospore expulsion in the dissemination of the chestnut blight fungus has been emphasized by various writers since the process was first observed by Rankin. A brief consideration of the work bearing on this phenomenon is presented in the historical introduction to a recent article[1] and will not be repeated here.

The effect of continued desiccation on the expulsion of ascospores has an important bearing on dissemination, since chestnut products bearing perithecial stromata are frequently transported for some distance. If these products are dried, the question has been raised as to how long the perithecia will retain their power to eject spores if subjected again to favorable conditions of moisture and temperature.

Anderson and Babcock[2] first brought out the fact by laboratory tests that expulsion of spores is resumed after desiccation if moisture is supplied. The longest period of drying recorded in this first work was seven days and this appeared to have little or no effect on the power of the perithecia to expel spores. In a later article by Anderson and Rankin[3], the influence of long desiccation is reported as follows: "The writers also found that the perithecia will retain their ability to eject spores for at least seven months under perfectly dry conditions." No detailed description is given of the tests on which this statement is based.

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[1] Heald, F. D. & Walton, R. C. The expulsion of ascospores from the perithecia of the chestnut blight fungus, *Endothia parasitica* (Murr.) Anders. Am. Jour. Bot. 1: 499–521. *f. 1, 2.* 1914.

[2] Anderson, P. J. & Babcock, D. C. Field studies on the dissemination and growth of the chestnut blight fungus. Bull. Pa. Chest. Tree Blight Comm. 3: 1–45. 1913.

[3] Anderson, P. J. & Rankin, H. W. The Endothia canker of the chestnut. Bull. Cornell Agr. Exp. Sta. 347: 531–618. 1914.

Alternate drying and drenching of cankers is, of course, the normal condition in the field. Work to be reported later has brought out the fact that under these conditions given areas of the bark of cankers will continue to expel ascospores through all or at least parts of two seasons.

METHOD

Bark showing an abundance of mature perithecial stromata was collected at Emilie, Pa., on July 15, 1913. The bark was then moistened and tested to make sure that the perithecia were in a condition to expel spores.[4] As soon as any specimens showed expulsion of spores, they were allowed to dry. By July 21, 1913, all the specimens to be retained had been tested and were thoroughly dry. They were then grouped in packets of six specimens each, giving to each lot as near as possible uniform material. They were then stored in a closed pasteboard box and retained in the laboratory.

Each of the six pieces of bark in a packet was of sufficient size to support an object glass. Tests were made at about monthly intervals employing six pieces each time. The method employed was that previously described by Heald and Walton.[5]

RESULTS

The results obtained by the different tests can best be presented in tabular form (Table I). From this summary it may be noted that the perithecia still retained the power to expel spores after 11 months and 18 days of continuous drying. In all probability the limit was not reached, as the perithecia seemed to develop as strong powers of spore expulsion during the last test as in any of the earlier ones. In the majority of cases all of the pieces of bark tested showed active ostioles after being subjected to favorable conditions for a sufficient length of time, and there was

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[4] The material was collected by Mr. R. C. Walton and some of the earlier tests were made by him.

[5] Loc. cit.

TABLE I

SUMMARY OF EFFECT OF DESICCATION ON ASCOSPORE EXPULSION

Period of Desiccation	No. of Pieces Showing Expulsion	Day of Test on Which 1st Expulsion Occurred	Day of Test on Which the the Last Trap Began to Show Active Ostioles	Maximum No. of Active Ostioles per Day on Any Trap	Duration of Test
1 mo., 13 days	5	2	11	100+	1 mo., 12 days
2 " 3 "	6	2	26	72	1 " 15 "
3 " 3 "	6	19	24	200+	1 " 3 "
4 " 7 "	5	21	58	200	2 " 18 "
5 " 9 "	6	23	54	200+	2 " 2 "
6 " 13 "	5	30	39	200	1 " 28 "
8 " 3 "	5	38	48	40	2 " 3 "
8 " 17 "	6	30	38	300+	1 " 20 "
9 " 14 "	6	20	29	250+	1 " 6 "
10 " 14 "	6	17	22	200+	1 "
11 " 18 "	6	18	21	250+	1 "

never more than one in any set in which no active ostioles were present. After one and two months of drying, certain perithecia began to expel spores on the second day of the test, but with longer desiccation the initiation of the process was considerably delayed, the number of days required varying from 17 to a maximum of 38. Even during the early part of the desiccation period the beginning of spore expulsion was considerably delayed in some of the specimens. The day of the test on which the last

TABLE II

RECORD OF TYPICAL SPECIMENS SHOWING CHARACTERISTIC INCREASE IN THE ACTIVITY OF SPORE EXPULSION

No. of Day	Nos. of Specimens and the Number of Active Ostioles on Successive Days							
	339	345	347	373	384	387	393	394
1	4	3	3	7	2	10	15	12
2	5	4	2	24	20	30	18	30
3	11	9	6	50	30	90	42	52
4	13	32	16	85	60	250	80	78
5	28	35	23	80	65	250+	100	80
6	41	154	31	175	75	"	130	60
7	60	158	46	300	100	"	150	65
8	69	178	47	300+	100+	"	100	15
9	89	150	54	"	"	"	"	15
10	112	193	58	"	"	"	"	14
11	97	200+	63	"	150	"		17
12	68	200+	67	"	150+	"		13
13	119	"	61	"	"	"		
14	172	"	73	"		"		
15	126	"	76	"		"		

specimen showing expulsion began its activity varied from 11 to 58 days.

Many of the specimens showed a marked and characteristic increase in the number of active ostioles after the beginning of spore expulsion. There appears to be a gradual increase to a maximum of activity with more or less fluctuation after the maximum has been reached. This peculiarity is illustrated by the record for a number of typical specimens as presented in the accompanying table (Table II). In the majority of cases the record was not continued beyond the maximum of activity.

THE VIABILITY OF THE SPORES EXPELLED FROM DESICCATED PERITHECIA

The longevity of ascospores of the chestnut blight fungus has been reported from tests made on material stored under different conditions. According to Anderson,[6] ascospores ejected from perithecia on to glass slides continued viable for five months and six days, while Anderson and Rankin[7] report that spores taken directly from perithecia in desiccated bark retained the power to germinate for a much longer period. Their statements on this point are as follows: "At the end of one year very little diminution in the percentage of germination could be seen." "In another series of experiments a large percentage of ascospores similarly stored germinated after eighteen months." No germination tests were reported of spores expelled from desiccated perithecia, but judging from their results on spores taken direct from dried material, one would expect a good germination of spores expelled after a prolonged drying of the bark.

During the progress of the work on spore expulsion reported in this paper, germination tests of the expelled spores were made. In each determination the percentage of viable spores was determined in duplicate cultures by the hanging-drop method. A spore print from a given specimen was covered with sterile water and rubbed up with a sterile loop to secure a uniform suspension, and the culture made by transferring spores from this suspension to dextrose agar. The results of these germination tests are shown in Table III. The slight reduction in germination percentage is in agreement with the results reported by Anderson and Rankin for ascospores under the conditions of their tests.

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[6] Anderson, P. J. The morphology and life history of the chestnut blight fungus. Bull. Pa. Chestnut Tree Blight Comm. 7: 1–44. 1913.

[7] Loc. cit.

TABLE III

Germination Percentages of Ascospores Expelled from Desiccated Bark

Specimen Number	Period of Desiccation	Germination Percentages	Average Germination Percentages
341	5 mo., 9 days	98	98.25
341	" "	98.5	
345	5 mo., 9 days	86.75	86.27
345	" "	85.8	
349	6 mo., 13 days	59	61
349	" "	63	
350	6 mo., 13 days	81.5	82.25
350	" "	84	
373	8 mo., 17 days	69.7	69.3
373	" "	68.9	
392	10 mo., 14 days	50	36.58
392	" "	29.16	
393	10 mo., 14 days	78	77.9
393	" "	77.8	

Conclusions

Continued desiccation does not inhibit the power of the perithecia of *Endothia parasitica* to expel spores when subjected to favorable conditions of temperature and moisture. It does, however, lengthen the period from the beginning of favorable conditions to the first expulsion. On account of this fact, it does not seem probable that perithecial material which has been retained in a dry condition for three months or more would ever be subjected under natural conditions to favorable influences for a sufficient length of time to induce spore expulsion. Material dried for one or two months, might, however, be a source of danger, as more or less expulsion might be induced by natural agencies.

Spores expelled from desiccated perithecia show little or no reduction in the percentage of germination.

It seems probable from our results and from those of others already cited that the time limit of ability to expel ascospores was not reached in the tests here recorded.